"STUDIES ON PREVENTION OF RHEUMATOID ARTHRITIS BY USING SELECTED PLANTS"

Synopsis submitted to

SWAMI RAMANAND TEERTH

MARATHWADA UNIVERSITY, NANDED

For the degree of

DOCTOR OF PHILOSOPHY

In the subject of

Herbal Medicine

Under the faculty of

SCIENCE

By

Dhole Gajanan Arjunrao

Under the guidance of

Dr. S. S. Bodke

Associate Professor & Head,

Department of Botany and Horticulture,

Yeshwant Mahavidyalaya, Nanded-431602.

Title: "Studies on prevention of Rheumatoid Arthritis by using selected plants"

Abstract of proposed work plan/ problem:

Rheumatoid arthritis is a chronic inflammatory joint disease, which can cause cartilage and bone damage. These conditions progress which progress to joint destruction, bone erosions and leading to swelling and inflammations. The pathogenesis of disease and the mechanisms are fully known such as inhibition of cyclooxygenase, xanthine oxidase, anti-proteolytic activity, β-glucornidase and hydroperoxides etc. and other various enzymatic targets. Free radical mediated pathogenesis also have important role in the initiation and progression of rheumatoid arthritis. Potentially, any organ and tissue affected by this degenerative disease and the increased risk of the major complication responsible for a worse prognosis. The aim of this study to inhibit various therapeutic targets using traditionally used plants for prevention and management of rheumatoid arthritis.

1. Introduction:

Rheumatoid arthritis is a chronic inflammatory systemic disease which is variable in its effects but can progress swiftly to become severe and disabling in a short period of time. The disease primarily affects the synovial joints, resulting in pain, deformity and eventual functional limitation, causing substantial morbidity and accelerated mortality. The condition also has widespread extra articular manifestations including vasculitis, inflammation in the heart and lungs and peripheral neuropathy. The disease confers an increased risk of many other diseases including cardiovascular disease, pulmonary dysfunction, renal disorders and intestinal pathologies, along with a significantly increased risk of premature death (Jonsson, 2008).

Rheumatoid arthritis is a chronic inflammatory disease, producing joint damage mediated by cytokines, chemokines, and metalloproteases. It is speculated that rheumatoid arthritis is a relatively new disease because there is a surprising lack of historical evidence for its existence (Hansen, 1995). The disease is systemic, characteristically affecting the synovial joints and periarticular synovial structures. This typically starts with the small bones of the hands and feet, although any joint can be involved. In advanced cases of the condition, most joints are become affected. The systemic nature of the condition means that many other organs may become involved as the condition progresses. Examples of extra-articular involvement can include symptoms and effects such as fever, weight loss, fatigue or weakness, swollen lymph nodes,

anemia, nodules, dry eyes, fibrosis of the lungs, fluid in the chest cavity, vasculitis, neuropathy, and kidney disease.

The main joint pathology in rheumatoid arthritis is an auto-immune mediated thickening and inflammation of the synovial membrane, which becomes infiltrated with inflammatory cells. With time this results in degeneration of the cartilage and the joint which leading to more inflammation and pain. The condition is variable in its initial presentation. Symptoms can occur either as a single episode of stiff and painful joints which may last some months, or as an aggressive and destructive condition which progresses rapidly, and if unchecked, leads to severe physical disability (Young, 2000).

By 2015, 23.7 million adults reported activity limitation due to their effect of arthritis in United States. By 2040, the number of US adults with doctor-diagnosed arthritis is projected to increase 49 percent to 78.4 million (25.9 percent of all adults), and the number of adults with arthritis-attributable activity limitation will increase 52 percent to 34.6 million (11.4 percent of all adults) (Hootman 2016).

Rheumatoid arthritis is one of the most common diseases in India. India is a low/middle income country and the population prevalence of rheumatoid arthritis in India is 0.75%. It was estimated that 9 million people could be affected with arthritis (Misra et al., 2017). The medicinal plants are rich source of bioactive compounds. They will be used as new anti-inflammatory herbal drugs for prevention of rheumatoid arthritis.

Considering all these facts the topic entitled "Studies on prevention of Rheumatoid Arthritis by using selected plants" is selected for the proposed research work.

2. Review of Literature and Development in the subject (Previous work done in the relevant area):

The several plants and their extracts were used for the in-vitro and in-vivo anti-inflammatory activities of different solvent extracts and their free radical scavenging potentials. Following are the few representative reports as follows. The sequentially extracted plant samples as, *Cissus quadrangularis*, *Plumbago zeylanica*, *Terminalia bellarica* and *Terminalia chebula* in water, ethanol and hexane extract showed in-vitro cyclooxygenase (1 & 2) inhibitory and antioxidant activities. The extracts of *T. bellarica* (73.3 4 %) and *T. chebula* (74.81 %) showed significant cyclooxygenase-2 inhibition as compared to other samples. The ethanol extract of the

selected plants demonstrated effective DPPH, OH and superoxide radical scavenging activity. (Shaikh et al., 2016). The maximum amount of inhibition of protein denaturation was showed in hydro alcoholic extract of stem of *P. murex*. The hydro alcoholic extract of *P. murex* possesses anti- inflammatory activity (Godhandaraman & Ramalingam, 2016). Preliminary phytochemical analysis of ethanol extract of *Hugonia mystax* showed the presence of carbohydrates, flavonoids, steroids, tannins, saponins, terpenoids and absence of alkaloids, proteins, amino acids. *Hugonia mystax* leaf possesses significant anti-inflammatory activity. (Vijayabaskaran & Kumar, 2016). The fruits of *Barringtonia racemosa* are traditionally used in Indian medicine for the treatment of pain and inflammatory conditions. Ethyl acetate extract of fruits of *B. racemosa* were showed for anti-inflammatory activity in experimental models of acute and chronic inflammation. (Patil & Patil, 2017). Several species of *Bridelia* have been used in the condition of pain & arthritis in Indian folk medicine. Extracts exhibited significantly inhibit mean paw edema of rats induced by carrageenan & histamine at dose of 200 & 400 mg/kg in-vivo (Tatiya et al., 2017). The *Smithia sensitiva* used as an anti inflammatory and antioxidant drug by tribal peoples in kerala. These methods showed significant anti-inflammatory property of the methanolic extract. (Sreena & Nair, 2016). Hydroalcoholic extract and its ethyl acetate fraction of *Hemidesmus indicus* showed significantly higher anti-arthritic activity than chloroform and residual other fractions (Mehta et al., 2012).

3. Objectives of Proposed Research Work:

- To study antioxidant activity of plant extracts
- To study xanthine oxidase inhibitory activity of plant extracts.
- To study anti-proteolytic activity of selected plant samples.
- To study the inhibition of hydroperoxides by plant extracts.
- To study beta-glucornidase inhibitory activity of plants extracts.
- To study cyclooxygenase enzyme inhibitory activity of plant extracts.
- To study cytotoxicity of plant extracts on normal cell lines.
- Isolation and identification of novel bioactive compounds from most active plant extracts

4. Methodology to be adopted:

1. The collected plant samples will be dried and will be prepare to fine powder. The individual samples will be sequentially extracted. The obtained extracts will be collected and will be maintained at 4°C for further experiments.
2. Antioxidant activity of the plant extracts will be screened using superoxide (Francisco et al., 2012) and DPPH (Ramzi and Mothana, 2011), hydroxyl (Sabir et al., 2012), nitric oxide radical method (Konrath et al., 2012).
3. The xanthine oxidase inhibitory activity of plant extracts will be determined by (Umamaheswari et al., 2009).
4. An anti-proteolytic activity of plant samples will be determined by Tandon et al. (1982). The amount of protein will be estimated by Lowry et al. (1951).
5. Membrane solution will be prepared by Dodge et al. (1963) with modification in buffer (Quist, 1980). Plant extracts will be evaluated for inhibition of hydroperoxides.
6. The effect of the plant extracts on activity of β-glucuronidase will be studied using a method described by Demetrios et al., (1998).
7. The cyclooxygenase inhibition assay will be performed by using method (Maurias, 2004).
8. The MTT cytotoxicity assay will be performed by (Carmichael et al., 1987).
9. Active extracts will be further fractionated using column chromatography (Choi et al., 2008). Later on these isolated fractions will be identified and characterized using various techniques.

5. Importance of study/ Society application:

- **International status:**

  By 2015, 23.7 million adults reported activity limitation due to their effect of arthritis in United States. By 2040, the number of US adults with doctor-diagnosed arthritis is projected to increase 49 percent to 78.4 million (25.9 percent of all adults), and the number of adults with arthritis-attributable activity limitation will increase 52 percent to 34.6 million (11.4 percent of all adults) (Hootman 2016).

- **National Status:**

  Rheumatoid arthritis is one of the most common diseases in India. India is a low/middle income country and the population prevalence of rheumatoid arthritis in India is 0.75%. It was estimated that 9 million people could be affected with arthritis (Misra et al., 2017).

- **Significance of the study:**

  The results of the proposed research work may useful for the preparation of effective herbal medicine. Isolated active extracts from medicinal plants will be used as new anti-inflammatory formulations for prevention of rheumatoid arthritis.

6. Proposed work Plan :

- **Year-wise Plan of work and targets to be achieved**

Time Duration	**Work plan**
First Year	➢ Collection of plant samples and drying. ➢ Extraction of plants in different solvents using Soxhlet apparatus. ➢ To study antioxidant activity of plant extracts. ➢ To study xanthine oxidase inhibitory activity of plant extracts. ➢ To study anti-proteolytic activity of selected plant samples.
Second Year	➢ To study the inhibition of hydroperoxides by plant extracts. ➢ To study β-glucoronidase inhibitory activity of plants extracts. ➢ To study cyclooxygenase inhibitory activity of plant extracts. ➢ To study cytotoxicity of plant extracts on normal cell lines.
Third Year	➢ Active extracts will be fractionated using chromatography. The isolated fractions will be identified and characterized using various techniques. ➢ The activity of bioactive compounds will be evaluated using different parameters.

7. References:

1. Hansen S.E. (1995). 'William Musgrave's (1655-1721) system of arthritides. Did it include rheumatoid arthritis?' *Scand J Rheumatol.* 24(5):274-8
2. Jonsson, B., (2008). 'Patient access to rheumatoid arthritis treatments. *Europ. J. of Health Econ.*, 8 (2):S35–S38.

3. Young, A. et al., (2000). How does functional disability in early rheumatoid arthritis (RA) affect patients and their lives? Results of 5 years of follow-up in 732 patients from the Early RA Study (ERAS). *Rheumatol*, 39, 603–611.
4. Shaikh R. U., Pund M. M., Gacche R. N., (2016). Evaluation of anti-inflammatory activity of selected medicinal plants used in Indian traditional medication system in vitro as well as in vivo. *J. of Tradit. & Complem. Med*. 6: 355- 361.
5. Godhandaraman S., Ramalingam V.,(2016). In vitro anti-inflammatory activity of different parts of *Pedalium murex* (L.). *Inter. J. of Herb. Med.*, 4(3): 31-36.
6. Vijayabaskaran., M, Kumar S., R.,(2016). Anti-Inflammatory activity of ethanol extract of *Hugonia mystax* Leaves. *J. of Pharm. and Phytochem.*, 5(4): 227-229.
7. Patil K. R., Patil C.R.,(2017). Anti-inflammatory activity of bartogenic acid containing fraction of fruits of *Barringtonia racemosa* Roxb. in acute and chronic animal models of inflammation. *J of Trad. & Complem. Med.* (7) 86- 93.
8. Tatiya A.U., et al., (2017). Evaluation of analgesic and anti-inflammatory activity of *Bridelia retusa* (Sp reng) bark. *J of Trad. & Complem. Med.* (7),4 41-451.
9. Sreena K.,Nair S. S., (2016). Evaluation of anti-inflammatory activity of the plant extract *Smithia Sensitiva*. *Der Pharmacia Lettre*, 8 (4):310-314.
10. Mehta et al., (2012). Anti-arthritis activity of roots of *Hemidesmus indicus* R.Br. (Anantmul) in rats. *Asian Pacif. J. of Trop. Med.*,130-135.
11. Francisco, C.H. etal., (2012). Evaluation of the antioxidant potential of cauliflower (Brassica oleracea) from a glucosinolate content perspective. *Food chem.* 132, 1003-1009.
12. Ramzi, A.A., Mothana, S.A., (2011). Anti-inflammatory, antinociceptive and antioxidant activities of the endemic Soqotraen *Boswellia elongata* Balf. f. and *Jatropha unicostata* Balf. f. in different experimental models. *Food and Chem. Toxicol.* 49, 2594-2599.
13. Sabir, et al., (2012). Antioxidant and hepatoprotective activity of ethanolic extract of leaves of Solidago microglossa containing polyphenolic compounds. *Food chem.* 131, 741-747.
14. Konrath et al., 2012. Investigation of the in vitro and ex vivo acetylcholinesterase and antioxidant activities of traditionally used Lycopodium species from South America on alkaloid extracts. *J. of Ethanopharm.* 139, 58-67.
15. Umamaheswari et al.,(2009). In vitro xanthine oxidase inhibitory activity of the fractions of *Erythrina stricta* Roxb. *J. of Ethanopharm.*124, 646–648.

16. Tandon et al., (1982). Anti-inflammatory and anti-proteolytic activities of newer indolyl-isoquinilines. *Drug Res.* 32, 1233-1235.
17. Lowry et al., (1951). Protein measurement with folin phenol reagent. *J. of Bio. Chem.* 193, 265-75.
18. Dodge et al., (1963). The preparation and chemical characteristics of Hb-free ghosts of human erythrocytes. *Arch. of Biochem. and Biophy.* 100, 119-130.
19. Quist, E.E., (1980). Regulation of erythrocyte membrane shape by Ca2+. *Biol. and Biophy. Res. Comm.* 92, 631-637.
20. Demetrios et al., (1998). Synthesis and biological evaluation of several coumarin-4-carboxamidoxime and 3- (coumarin-4yl)-1, 2, 4-oxadiazole derivatives. *Euro. J. of Med. Chem.* 33, 715-724.
21. Maurias, M., (2004). Resvertrol analogues as selective cyclooxygenease-2 inhibitors: synthesis and structure activity relationship. *Bioorg and Med. Chem.* 12, 5571-5578.
22. Carmichael et al., (1987). Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Can. Res.* 47, 936–942.
23. Choi et al., (2008). Glycation inhibitory activity and the identification of an active compound in *Plantago asiatica* extracts. *Phytothe. Res.* 22, 323-329.
24. Hootman, (2016). Arthritis By The Numbers, Book of Trusted Facts & Figures. Arthritis Foundation, 1-99.
25. Misra et al., (2017). 2016 update of the EULAR recommendations for the management of rheumatoid arthritis: autopia beyond patients in low/middle income countries? *Ann Rheum Dis.*,1–2.

Signature	Signature
(Dhole Gajanan Arjunrao)	(Dr. S. S. Bodke)
Research Scholar	**Research Supervisor / Guide / Co-guide**